

Attorney Docket No.: **PTQ-0027**
Inventors: **Van Eyk et al.**
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Amendments to Specification:

Please replace the paragraph beginning at page 5, line 25 with the following:

Figure 3 shows the results of an SDS-PAGE analysis of reperfusion effluent. Reperfusion effluent was collected from rat hearts which had undergone 15 min equilibration followed by 60 min of ischemia. Panel A shows the 12.5% SDS polyacrylamide gel stained with coomassie blue of the two minute effluent fractions ~~collect~~ collected at 0 and 2 min. Serum albumin and triose phosphate isomerase were identified by amino acid sequencing (Table 2). Panels B to F show the western blots of the combined effluent fractions (0 to 4 min) probed with anti- α -actinin (panel B), anti-TnT (panel C), anti-tropomyosin (TM) (panel D), anti-TnI peptide P142T (residues 136 to 148; SEQ ID NO:34) (MAb E2, panel E) and anti-MLC1 (panel F) antibodies. The MLC1 modification product is indicated by an arrow.

Please replace the paragraph beginning at page 6, line 5 with the following:

Figure 4 shows the results of an SDS-PAGE analysis of skinned left ventricle tissue samples from isolated rat hearts. Tissue samples obtained from hearts which experienced 15 min equilibration followed by either 45 min perfusion (control, 1),

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15 min ischemia followed by 45 min reperfusion (i.e., 15/45; 2), 60 min ischemia (3) or 60 min ischemia followed by 45 minutes reperfusion (i.e., 60/45; 4) were skinned in 50% glycerol prior to being prepared for SDS-PAGE analysis. Panel A shows the coomassie blue stain of the 12.5% crosslinked gel. Panels B-F show corresponding western blots using anti- α -actinin (panel B), anti-TnI peptide residues 136 to 148 (SEQ ID NO:34) (MAb E2, panel C), anti-TnT (panel D), anti-TM (panel E), and anti-MLC1 (panel F) antibodies. Panel G shows the western blot of a 10% SDS-PAGE of control tissue and tissue obtained from rats which experienced 60 min ischemia (2). The western blot was probed with anti- α -actinin antibody. Modification products are indicated by arrows.

Please replace the paragraph beginning at page 7, line 1 with the following:

Figure 6 shows the results of an SDS-PAGE analysis of isolated myofibrils from control and globally ischemic rat hearts. Left ventricular tissue samples obtained from isolated rat hearts were placed in saline in plastic bag for 60 min at either 4°C (control, 1) or 39°C (global ischemia, 2). Panel A shows the coomassie blue stain of the 12.5% crosslinked gel. Panels B to F show corresponding western blots using anti- α -

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actinin (panel B), anti-TnI peptide residues 136 to 148 (SEQ ID NO:34) (panel C), anti-TnT (panel D), and anti-MLC1 (panel E) antibodies. Modification products are indicated by arrows. The data reveal a loss of α -actinin in the global ischemic myofibrils and degradation of TnI and MLC1, respectively.

Please replace the paragraph beginning at page 7, line 9 with the following:

Figure 7 shows an immunological analysis of the troponin I modification product. Figure 7A shows a western blot of left ventricular tissue samples obtained from hearts which experience 60 min ischemia followed by 45 min reperfusion (ischemic tissue) using the anti-troponin I antibodies E2 and 10F2. Figure 7B shows a western blot of MAb 10F2 against intact cardiac troponin I (lane 1), troponin I peptide residues 129-175 (SEQ ID NO:35) (lane 2), troponin I residues 54 to 210 (SEQ ID NO:36) (lane 3), troponin I residues 1 to 188 (SEQ ID NO:37) (lane 4) and troponin I residues 1 to 199 (SEQ ID NO:38) (lane 5). The 22 kDa TnI degradation product has C-terminus proteolysis.

Please replace the paragraph beginning at page 9, line 21 with the following:

Figure 16 shows a western blot of myocardial biopsy tissue samples taken before and following coronary bypass surgery in two

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human patients (panels A and B), and shows TnI modification. the biopsy samples from the left (LV) and right ventricles (RV) were immediately frozen in liquid nitrogen before the cross clamp was positioned (before) and 10 minutes following removal of the crossclamp (after). Proteins were separated by 12% SDS-PAGE and transferred to nitrocellulose for western blot analysis using the anti-cardiac TnI MAb 8I-7 (epitope TnI amino acid residues ~~188 to 199~~ 137 to 148 (SEQ ID NO:47).

Please replace the paragraph beginning at page 10, line 1 with the following:

Figures 17A to D show complete amino acid sequences for human TnI (SEQ ID NO:8; A), rat TnI (SEQ ID NO:11; B), human TnT (SEQ ID NO:14; C) and rat TnT (SEQ ID NO:17; D) and the literature references therefor. These sequences are also compiled and are available at the website (<http://genome.with.the.extension.cs.unc.edu> of the world wide web).

Please replace the paragraph beginning at page 10, line 21 with the following:

The phrase "myofilament protein modification product(s) is intended to include one or more modification products of a myofilament protein associate with damage to the myocardium or skeletal muscle. For example, a myofilament protein modification

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produce can be a modified form of the protein or a peptide fragment of a myofilament protein such as α -actinin, a troponin (e.g., troponin I, troponin T), or myosin light chain 1. Examples of such peptide fragments include all or a portion of the carboxyl-terminal region consisting of amino acids 194-210 (rat sequence, see Figure 17B, SEQ ID NO:26; corresponding human sequence, see Figure 17A, SEQ ID NO:27) of troponin I, or all or a portion of the amino-terminal region consisting of amino acids 1 to 193 of troponin I (rat sequence, SEQ ID NO:20; corresponding human sequence, SEQ ID NO:21) (referring to the sequence published in any one of Vallins et al. 1990, FEBS Lett. 270:57-61; Armour et al. 1993, Gene, 131:287-292; or Hunkeler et al. 1991, Circ. Res. 69:1409-14). Alternatively, a myofilament protein modification product can be a peptide fragment of myosin light chain 1, such as all or a portion of all the carboxyl-terminal region consisting of amino acids 20 to 199 192 (SEQ ID NO:28) of myosin light chain 1, or all or a portion of the amino-terminal region consisting of amino acids 1 to 19 (SEQ ID NO:29) of myosin light chain 1 (referring to the sequence published in Zimmermann et al. 1990, J. Mol. Biol. 211(3):505-513). A myofilament protein modification product can be a covalent or non-covalent complex of two or more intact proteins or fragments

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of proteins, such as α -actinin, troponin I, T, or C, or myosin light chain 1, or covalent or non-covalent complexes of these proteins or fragments thereof with other proteins or fragments thereof. A myofilament protein modification product can also be such a complex of peptide fragments of two or more of α -actinin, troponin I, T or C, or myosin light chain 1, or such complexes of these proteins with other proteins or fragments thereof. Such complexes include those formed from any combination of the three troponins (troponin I, T and C), or fragments thereof such as, for example: TnI (amino acids 1 to 193; rat sequence, SEQ ID NO:20; corresponding human sequence, SEQ ID NO:21) with TnT (amino acids 191-298; rat sequence, SEQ ID NO:30; corresponding human sequence, SEQ ID NO:32); and TnI (amino acids 1 to 193; rat sequence, SEQ ID NO:20; corresponding human sequence, SEQ ID NO:21) with TnC (SEQ ID NO:48) (amino acids 1 to 94 (SEQ ID NO:49) (see Table 4).

Please replace the paragraph beginning at page 12, line 4, with the following:

Ischemia/reperfusion injury ranges from mild to severe. The terms "mild ischemia" and "mild ischemia/reperfusion injury" refer to situations in which reversible damage to skeletal muscle or the myocardium has occurred. In these situations, the muscle

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can eventually regain the ability to contract and a full recovery is possible. Usually, in such situations, the majority of the cells comprising the affected muscle retain integrity of the cellular membrane. Mild myocardial ischemia and/or ischemia/reperfusion injury are marked by the presence of one or more of a cardiac troponin I modification product(s) (e.g., amino acid residues 1 to 193; rat sequence, SEO ID NO:20; corresponding human sequence, SEO ID NO:21), the loss of α -actinin, and the formation of covalent and/or non-covalent complex(es). It is expected that mild ischemia or ischemia/reperfusion injury of skeletal muscle will demonstrate similar myofilament protein modification products.

Please replace the paragraph beginning at page 12, line 14, with the following:

The terms "severe ischemia" and "severe ischemia/reperfusion injury" refer to situations where irreversible damage to skeletal muscle or the myocardium has occurred, i.e., situations where the muscle cannot regain its full ability to contract. Usually, in such situations, there is a loss of cellular membrane integrity and cellular proteins are released and necrosis occurs. Severe myocardial ischemia and/or ischemia/reperfusion injury are often marked by the presence of one or more of a myosin light chain 1

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modification product(s) (e.g., amino acid residues 20 to 199
192 (SEQ ID NO:28)), an additional TnI modification product(s)
(e.g., amino acid residues 63 to 193; rat sequence, SEQ ID NO:22;
corresponding human sequence, SEQ ID NO:23, amino acid residues
73 to 193; rat sequence, SEQ ID NO:24; corresponding human
sequence, SEQ ID NO:25), TnT modification product(s), and a-
actinin modification product(s).

Please replace the paragraph beginning at page 14, line 3,
with the following:

Assessment of myocardial or skeletal muscle damage in a
biological sample can be performed by direct detection of
myofilament protein modification product(s) in the sample, using,
for example, chromatography techniques such as HPLC, or
electrophoresis. These analyses are used to detect differences
between elution profiles of samples obtained before and after,
for example, treatment of hypoxemia, hypoxia, ischemia or
ischemia/reperfusion. As well, the appearance or disappearance
of one or more myofilament protein modification products,
peptides, or fragments, such as, for example, cardiac TnI
residues 194 to 210 (rat sequence, SEQ ID NO:26; corresponding
human sequence, SEQ ID NO:27) or myosin light chain residues 1 to
199 192 (SEQ ID NO:28), in the elution profiles obtained during

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HPLC analysis can be used as indicators of muscle damage.

Please replace the paragraph beginning at page 14, line 20,
with the following:

As used herein the term "compound" is intended to include any agent which specifically recognizes and binds to an intact myofilament protein and/or a modification product thereof as defined herein. For example, the compound can be an antibody, a target protein, a peptide or a peptidomimetic, either synthetic or native, labeled or unlabeled. The term "specifically binds" means binding to a particular intact myofilament protein (e.g., troponin I) and/or a modification product thereof (e.g., cardiac TnI residues 1 to 193 (rat sequence, SEQ ID NO:20; corresponding human sequence, SEQ ID NO:21), covalent complex comprising myofilament fragments such as TnI residues 1 to 193 (rat sequence, SEQ ID NO:20; corresponding human sequence, SEQ ID NO:21) with TnT residues 191 to 298 (rat sequence, SEQ ID NO:30; corresponding human sequence, SEQ ID NO:32), or covalent complex comprising, for example, intact TnI and TnT, such as a 66 kDa complex found in skeletal muscle or human cardiac biopsy) without substantially binding to any other intact myofilament protein and/or a modification product thereof present in the biological sample. The term "antibody" as used herein encompasses all forms

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of antibodies known in the art, such as polyclonal, monoclonal, chimeric, recombinatorial, single chain and humanized antibodies, as well as functional fragments thereof (e.g., $F(ab')_2$ fragments), either synthetic or native, labeled or unlabeled, which specifically bind to a myofilament protein modification product. Binding between the compound and the myofilament protein modification product can be covalent or, preferably, non-covalent. When the myofilament protein modification product is a covalent complex, the compound can be a recombinant, native, or synthetic peptide or fragment thereof that recognizes a region or a portion of a region of the complex corresponding to the covalent bond.

Please replace the paragraph at page 23, beginning at line 1 with the following:

For example, when qualitatively characterizing different myofilament proteins and/or modification products present in the biological sample, antibodies can be used which differentially recognize epitopes present in the various modification products. Using a label that has a measurable moiety attached to it (e.g., β -galactosidase), a profile or "fingerprint" of the proteins and modification products can be obtained. This profile, which is expected to include, for example, characteristic ratios of

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various proteins and/or fragments from the same (e.g., cardiac TnI residues 1 to 193 of SEQ ID NO:8 (rat sequence, SEQ ID NO:20; corresponding human sequence, SEQ ID NO:21) vs. cardiac TnI residues 63 to 193 of SEQ ID NO:8 of (rat sequence, SEQ ID NO:22; corresponding human sequence, SEQ ID NO:23)) from different (e.g., TnI vs. myosin light chain I) proteins, can then be associated with a level (i.e., extent) or type of myocardial damage.

Please replace the paragraph at page 23, beginning at line 10 with the following:

Different myofilament proteins and/or modification products present in the biological sample can also be quantitatively characterized (e.g., compared to a standard). For example, levels of different troponin I modification products (e.g., a cardiac troponin I fragment consisting of amino acids 1 to 193 of SEQ ID NO:8 (rat sequence, SEQ ID NO:20; corresponding human sequence, SEQ ID NO:21)) can be compared to one another, or to levels of the intact troponin I protein, and this pattern of protein levels can be associated with a level (i.e., extent) or type of myocardial damage. Levels of myofilament proteins and/or modification products can be detected using for example quantifiable labels (e.g. antibodies labeled with an enzyme, the

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activity of which can be measured and correlated with levels of antibody binding), as are known in the art, which specifically bind to the proteins and/or modification products.

Please replace the paragraph at page 23, beginning at line 19 with the following:

In one embodiment, the method of the invention is used to diagnose mild ischemia by detecting the presence of skeletal or cardiac troponin I fragment (e.g., cardiac TnI residues 1 to 193 of SEQ ID NO:8 (rat sequence, SEQ ID NO:20; corresponding human sequence, SEQ ID NO:21)) and comparing the levels of this fragment to the levels of intact troponin I.

Please replace the paragraph at page 24, beginning at line 1 with the following:

For example, we describe herein that the extent and type of modification to TnI (amino acid residue 1 to 210 of rat TnI (SEQ ID NO:11); corresponding human sequence depicted in SEQ ID NO:8) change depending on whether mild or severe ischemic damage has occurred. With mild ischemia and/or ischemia/reperfusion, TnI is specifically degraded, yielding a fragment with apparent molecular weight of 22 kDa by SDS-PAGE, corresponding to amino acid residues 1 to 193 of SEQ ID NO:8 (rat sequence, SEQ ID NO:20; corresponding human sequence, SEQ ID NO:21), due to

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proteolysis of 16 amino acid residues form the C-terminus of TnI. In addition (or shortly thereafter), TnI 1 to 193 of SEQ ID NO:8 (rat sequence, SEQ ID NO:20; corresponding human sequence, SEQ ID NO:21) forms covalent complexes with TnC or TnT (32 kDa by SDS-PAGE). Later, with increasing severity of ischemic and/or ischemic/reperfusion damage, TnI is further degraded, yielding smaller fragments TnI 63 to 193 of SEQ ID NO:8 (rat sequence, SEQ ID NO:22; corresponding human sequence, SEQ ID NO:23) and 73 to 193 of SEQ ID NO:8 (rat sequence, SEQ ID NO:24; corresponding human sequence, SEQ ID NO:25) (16 and 15 kDa by SDS-PAGE).

Therefore, if a profile from a biological sample shows only a 22 kDa TnI protein fragment, rather than both a 22 kDa and a 16 kDa TnI fragment, the indication is that mild/reversible rather than severe/irreversible damage has occurred.

Please replace the paragraph at page 24, beginning at line 12 with the following:

Different myofilament proteins are more or less susceptible to modification depending on the extent of ischemic or ischemic/reperfusion injury that has occurred. Thus, the appearance of a certain modification to a specific proteins can be used as a marker/index for extent of muscle damage. For example, MLC1 degradation (residues 20-199 of SWISS-PROT

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~~Accession No. P17209 (<http://www.expasy.ch/cgi-bin/sprot-search-de> 192; SEQ ID NO:28)~~ occurs only with very severe ischemia in the myocardium. Therefore, if one detects this smaller fragment of MLC1 in a biological sample, it is an indication that the myocardium is severely and possibly irreversibly damaged.

Please replace the paragraph at page 24, beginning at line 26 with the following:

1. TnI degradation product residues 1 to 193 ~~of SEQ ID NO:8 (rat sequence, SEQ ID NO:20; corresponding human sequence, SEQ ID NO:21)~~ and loss of α -actinin.

Please replace the paragraph at page 24, beginning at line 27 with the following:

2. TnI or TnI 1 to 193 ~~of SEQ ID NO:8 (rat sequence, SEQ ID NO:20; corresponding human sequence, SEQ ID NO:21)~~ covalent complex formation. (As proteolysis and covalent complex formation may occur very rapidly the two species may thus be indistinguishable from one another.)

Please replace the paragraph at page 25, beginning at line 1 with the following:

3. TnI further degraded (Residues 63 to 193 ~~of SEQ ID NO:8 (rat sequence, SEQ ID NO:22; corresponding human sequence, SEQ ID NO:23)~~).

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Please replace the paragraph at page 25, beginning at line 2 with the following:

4. TnI further degraded (Residues 73 to 193 of ~~SEQ ID NO:8~~
(rat sequence, SEQ ID NO:24; corresponding human sequence, SEQ ID
NO:25)).

Please replace the paragraph at page 25, beginning at line 4 with the following:

6. MLC1 degradation (residues 20-~~199~~192(SEQ ID NO:28)).

Please replace the paragraph beginning at page 29, line 4, with the following:

Western blot analysis was done according to Van Eyk et al. 1998 (*Circ. Res.* 82:261-71) or else the primary antibodies were detected with goat anti-mouse IgG conjugated to alkaline phosphatase (Jandel Scientific) and CDP-Star chemiluminescence reagent (NEN-Mandel). The monoclonal antibodies used were anti-TnT clone JLT-12 (Sigma Chemical Co., St Louis, Mo), anti- α -actinin clone EA-53, (Sigma) or anti- α -actinin clone 157 (provided by Spectral Diagnostics, Toronto, Canada), anti-MLC1 (provided by Abbott Laboratories, Chicago, IL) which recognizes amino acid residues 70 to 75 of ~~SWISS-PROT Accession No.~~
~~P17209(SEQ ID NO:19)~~(SEQ ID NO:40):, anti-TM (Sigma), anti-sarcomeric actin (Sigma), and anti-glyceraldehyde phosphate

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dehydrogenase (Cedarline Lab. Ltd, Canada). Several different anti-TnI antibodies were utilized: anti-TnI clone 3309 which recognizes amino acid residues 157 to 192 ~~of SEQ ID NO:11 (SEQ ID NO:41)~~ and clone AM-NI which recognizes TnI residues 1 to 65 ~~of SEQ ID NO:11 (SEQ ID NO:42)~~ (provided by Dr. J. Ladenson, Washington University St Louis, Mo.), anti-TnI clone 10F2 (MAb 10F2) which recognizes amino acid residues 189 to 199 ~~of SEQ ID NO:11 (SEQ ID NO:43)~~ (see epitope map Figure 8 in Van Eyk et al. 1998, *Circ. Res.* 82:261-71), antibody provided by Dr. C. Larue at Univ. Innsbruck Med. School, Austria, MAb C5 (Research Diagnostics, Flanders, NS), and our anti-TnI peptide (P143T) residues 137 to 148 ~~of SEQ ID NO:11 (SEQ ID NO:44)~~ (MAb E2). The production of the anti-TnI peptide monoclonal antibodies including MAb E2 has been described in Van Eyk et al. 1995 (*Prot. Sci.* 4:781-90). MAb E2 recognizes intact skeletal and cardiac TnI and cardiac TnI peptides containing amino acid residues 136 to 148 ~~of SEQ ID NO:11 (SEQ ID NO:34)~~ (data not shown). As well, anti-TnI antibodies MAb 8I-7 and 3I-35 (both Spectral Diagnostics, Toronto, Canada), and MAb C5 (Research Diagnostics, Flanders, NS), which recognize TnI amino acid residues ~~136 to 147~~ 137 to 148 (SEQ ID NO:47), 188 to 199 (SEQ ID NO:39), and 188 to 199 ~~of SEQ ID NO:11 (SEQ ID NO:39)~~, respectively, see

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McDonough *et al.* 1998, *Biophysical J.* 74:A354). Epitope mapping of these various antibodies was carried out by 12% SDS PAGE of intact cardiac TnI and various TnI fragments followed by western blot analysis as outlined above. Bovine cardiac TnI and rabbit skeletal TnI were purified by HPLC (Ingraham *et al.* 1988, *Biochemistry* 27:5891-98); recombinant rat cardiac TnI fragments 54 to 210 (SEQ ID NO:36), 1 to 188 (SEQ ID NO:37), and 1 to 199 ~~of SEQ ID NO:11~~ (SEQ ID NO:38) were provided by Dr. A Martin (Univ. Illinois at Chicago, Chicago, IL; Rarick *et al.* 1997, *J. Biol. Chem.* 272:26887-92), and the synthetic skeletal TnI peptide 96 to 142, which is equivalent to the cardiac peptide residues 129 to 175 ~~of SEQ ID NO:11~~ (SEQ ID NO:45), was prepared by solid-phase peptide synthesis (Tripet *et al.* 1997, *J. Mol. Biol.* 271:728-50).

Please replace the paragraph at page 31 beginning at line 23 with the following:

To identify the site of modification in troponin I, specific antibodies to the amino- and carboxyl-termini of troponin I were used to find out which antibodies bind to the different modification products. The antibodies MAb 10F2 (recognizes residues 188 to 199 ~~of SEQ ID NO:11~~ (SEQ ID NO:39)) and MAb 3350

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(2F6.6) (recognizes residues 28 to 54 of ~~SEQ ID NO:11~~ (SEQ ID NO:46)) were used (Van Eyk et al. 1998, *Circ. Res.* 82:261-71). The various modification products were run on either a 12% SDS-PAGE or 10% T-PAGE (described in Schagger et al. 1987, *Analytical Biochemistry*, 166:368-79). The proteins were transferred to nitrocellulose using a 10 mM CAPS buffer pH 11.0 for 16 h at 27 V (described in Towbin et al. 1979, *PNAS* 76:4350-54). The carboxyl-terminus is usually the first to be clipped (Figure 7), yielding residues 1 to 193 of ~~SEQ ID NO:11~~ (rat sequence, SEQ ID NO:20; corresponding human sequence, SEQ ID NO:21) (Figure 9), but in addition there are further modifications occurring at the amino-terminus with more severe ischemia (Figure 9). Further TnI degradation products were identified as listed in Table 4.

Please replace the paragraph at page 32 beginning at line 19 with the following:

Cardiac TnC was crosslinked to 3M Emphaze Resin (Pharmacia) according to the manufacturer's protocol. The anti-TnI MAb 8I-7 (Spectral Diagnostics) (epitope residues ~~136-147~~ 137 to 148 (SEQ ID NO:47)) was crosslinked to CNBr Sepharose (Pharmacia) according to the manufacturer's protocol. Both columns were equilibrated in 20 mmol/l Tris-HCl pH 7.4, 50 mmol/l potassium chloride, 1 mmol/l calcium chloride. Tissue homogenates were

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loaded onto the column (10 mg), and washed with 10 column volumes of buffer. Bound proteins were eluted with 65 mmol/l glycine-HCl, pH 3.1. Fractions of 1 ml were collected into tubes containing 0.86 mol/l 3-N-morpholino propano-sulfonic acid, pH 8.0 to neutralize pH. The lyophilized fractions were resuspended in 0.05% aqueous trifluoroacetic acid, and analyzed by reversed phase high performance liquid chromatography (RP-HPLC), on an analytical Zorbax C8 300SB reversed-phase column (4.6-mm internal diameter x 250 mm, Chromographic Specialists Inc). The HPLC system consisted of a Varian 9100 Autosampler, 9012 Solvent Delivery System, and 9065 Polychrom (Varian, Mississauga, Canada). The proteins were eluted using an AB solvent system, where solvent A was composed of 0.05% aqueous trifluoroacetic acid, and solvent B was composed of 0.05% trifluoroacetic acid in acetonitrile. The AB gradient consisted of an isocratic hold (100% buffer A) for 5 minutes followed by a 2% buffer B/min linear gradient at a flow rate of 1 ml/min. The peaks were collected, lyophilized, and analyzed by mass spectrometry, western blotting, and amino acid microsequencing.

Please replace the paragraph at page 33, beginning at line 8 with the following:

Traces from RP-HPLC and mass spectrometry analyses are shown

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in Figures 9 and 10. Panel B of Figure 9 shows the single peak obtained from electrospray mass spectrometry of the RP-HPLC peak shown in panel A, isolated from TnC by affinity chromatography. Analysis of the rat cardiac (rc) TnI amino acid sequence identified a single sequence of appropriate mass, rcTnI residues 1 to 193 ~~of SEQ ID NO:11 (rat sequence, SEQ ID NO:20;~~ corresponding human sequence, SEQ ID NO:21) (see Table 4) (intact rcTnI has 210 amino acid residues, see Figure 17B).